

CLAIMS

We claim:

1. An array composition comprising:

- a) a substrate with a surface comprising discrete sites; and
 - b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises:
 - i) a bioactive agent; and
 - ii) an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated;
- wherein said microspheres are distributed on said surface.

2. An array composition comprising:

- a) a substrate with a surface comprising discrete sites; and
- b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent and does not comprise an optical signature, wherein said microspheres are distributed on said surface.

3. A composition according to claim 1 or 2 further comprising at least one decoder binding ligand.

4. A composition according to claim 1 or 2 wherein said bioactive agents are nucleic acids.

5. A composition according to claim 1 or 2 wherein said bioactive agents are proteins.

6. A method of making a composition comprising:

- a) forming a surface comprising individual sites on a substrate;
- b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulation each comprising a bioactive agent and do not comprise an optical signature.

7. A method of making a composition comprising:

- a) forming a surface comprising individual sites on a substrate;
- b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising:
 - i) a bioactive agent; and
 - ii) an identifier binding ligand that will bind at least one decoder binding ligand such

that the identification of the bioactive agent can be elucidated

8. A method of decoding an array composition comprising

a) providing an array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said surface;

b) adding a plurality of decoding binding ligands to said array composition to identify the location of at least a plurality of the bioactive agents.

9. A method according to claim 8 wherein at least one subpopulation of microspheres comprises an identifier binding ligand to which a decoding binding ligand can bind.

10. A method according to claim 8 wherein said decoding binding ligands bind to said bioactive agents.

11. A method according to claim 8 wherein said decoding binding ligands are labeled.

12. A method according to claim 8 wherein the location of each subpopulation is determined.

13. A method of determining the presence of a target analyte in a sample comprising:

a) contacting said sample with a composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent and do not comprise an optical signature; wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and

b) determining the presence or absence of said target analyte.

14. A method of determining the presence of a target analyte in a sample comprising:

a) contacting said sample with a composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising:

1) a bioactive agent; and

2) an identifier binding ligand that will bind a decoder binding ligand such that

the identification of the bioactive agent can be elucidated;

wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres; and

b) determining the presence or absence of said target analyte.

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15. An array composition comprising:

a) a substrate with a surface comprising discrete sites;

b) a population of microspheres distributed on said sites and comprising at least a first and a second subpopulation, each subpopulation comprising a bioactive agent, wherein said first subpopulation comprises at least a first optical dye with a first pKa and said second subpopulation comprises at least a second optical dye with a second pKa, wherein said first and second pKas are different.

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16. A method of making a microsphere array comprising:

a) contacting a substrate with a surface comprising discrete sites with a solution comprising a population of particles; and

b) applying energy to said substrate or said solution, or both, such that at least a subpopulation of said particles associate onto sites.

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17. A method according to claim 16 wherein said discrete sites comprise wells.

18. A method according to claim 16 wherein said energy is in the form of agitation.